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Growth Hormone Therapy and Bone Mineral Density in Turner Syndrome

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In a previous report, preliminary data showed a significant reduction in cortical bone mineral density (BMD) in women with Turner syndrome that had been treated with GH compared with women with Turner syndrome that had not been treated. To clarify this point, we have investigated the effects of GH treatment at multiple sites in this case-control, cross-sectional study. There were 23 women per group, who were similar in age, height, body mass index, estrogen use, and ethnic makeup. Median age (range) at start and duration of GH treatment was 9 (3–17) and 5 (2–9) yr, respectively. GH-treated women had a slightly greater (~8%, $P = 0.03$) width of the radial shaft, but otherwise there were no significant differences between groups in bone dimensions or BMD at the distal radius, lumbar spine, or femoral neck. Furthermore, regression analysis in a linear model including independent variables of age, age at diagnosis, body mass index, presence of spontaneous puberty, and GH use confirmed that GH use did not contribute to variation in BMD. (*J Clin Endocrinol Metab* 89: 4886–4889, 2004)

SHORT STATURE AFFECTS approximately 95% of women and girls with Turner syndrome (TS), and treatment with recombinant human GH has been recommended for girls who are below the fifth percentile of the normal female growth curve (1). The effects of GH therapy on linear growth are well documented; GH accelerates growth during childhood and pubertal years and increases final height (2, 3). In contrast, there is conflicting evidence on the impact of GH therapy on bone mineral density (BMD) in TS; some studies suggest improvement of BMD (4, 5), some studies show no effect (6, 7), and others suggest a decrease in BMD related to GH treatment (8, 9). Significant limitations of the studies assessing the effect of GH therapy on bone density in TS are small numbers of study subjects and/or lack of matched, untreated control groups. Recently, we reported that women with TS have a selective reduction of cortical BMD at the radius, as measured by dual x-ray absorptiometry (DXA), which was independent of hypogonadism and hormone replacement therapy (9), and noted that a subset of women who had been treated with GH showed lower cortical BMD than those who had not. In the present study, we reevaluated the impact of GH treatment on cortical bone density in TS with data from more subjects. In addition, we extended our observations to include other skeletal sites, including lumbar spine, femoral neck (FN), and the total hip.

Abbreviations: AP, Anteroposterior; BMAD, bone mineral apparent density; BMC, bone mineral content; BMD, bone mineral density; DXA, dual x-ray absorptiometry; FN, femoral neck; RAD-1/3, one third proximal radius; RAD-UD, ultradistal radius; TS, Turner syndrome.

Subjects and Methods

Subjects

Data from 91 subjects with TS who were younger than 40 yr of age were available for analysis. All study subjects were participants in an ongoing, institutional review board-approved, genotype/phenotype study on TS and were recruited mainly through web site notices and had signed informed consents. Criteria for participation included euthyroid status and 50-cell karyotype showing more than 70% of cells with a missing or abnormal sex chromosome.

Forty of the subjects had been treated with GH for 2 or more years. The rest either had never been treated with GH (n = 43) or had received GH for less than 6 months (n = 8). Four of these eight, ages 9–13 yr, were included in the nontreatment group. GH-treated subjects were significantly younger (mean age, 19.8 ± 7.8 yr) compared with nontreated (mean age, 26.6 ± 10.7 yr; $P = 0.001$). To avoid the confounding effects of age and secular growth trends, we selected subjects for a case-control study following this algorithm: The study subjects were organized into two groups, GH treated (index subjects) and non-GH treated (control), in the order of their admission to the study. Each consecutive index subject was matched to the first control subject from the list who had the same age. If an exact age match could not be found, then the first control closest to the age of the index subject (within ±1 yr) was picked. If there was no control available in the ±1-yr age range, then the index subject was excluded from the analysis. Using this algorithm, we were able to select 23 subjects in each group. Of the 23 subjects in the nontreatment group, nine subjects were diagnosed within 1 yr before study entry, six subjects had been diagnosed after the age of puberty, five subjects had declined GH therapy, and in the remaining three subjects (ages 31, 32, and 35 yr) GH therapy had not been recommended by their physicians. The pure 45,X karyotype was slightly more prevalent (statistically nonsignificant) in the GH-treated group: 14 of 23 *vs.* nine of 23 ($P = 0.24$).

BMD measurement

We measured bone mineral content (BMC) and areal BMD by DXA (QDR-4500A; Hologic, Inc., Bedford, MA) at the following sites: one third proximal radius (RAD-1/3, a predominantly cortical site), ultradistal radius (RAD-UD; a predominantly trabecular site), anteroposterior (AP) lumbar spine at L1–L4, FN, and total hip. Whole-body scans allowed determination of the grams of whole-body BMC. From mea-

surement of BMD of an anthropomorphic spine phantom, the coefficient of variation of the instrument was less than 0.5% over the period of the study. All scans were reviewed by a nuclear medicine physician. To ameliorate the confounding effect of bone size on the areal bone density measurement, we transformed the areal bone density (BMD) into volumetric (apparent) BMD (BMAD) for AP lumbar spine at L1–L4 (10), RAD-1/3, and RAD-UD (11) as previously described. We also recorded history of estrogen replacement, spontaneous puberty, treatment with thyroid hormones, treatment with androgens, and history of fractures.

Statistical methods

Data are presented as mean ± SD or as proportions. We compared means of continuous variables by one-way analysis of covariance with protected least significant difference *t* test and proportions by Z test for proportions. Continuous variables were log transformed to make the data distribution closer to the normal distribution. Multiple regression analysis was performed using the best subset regression model to evaluate the combined influence of GH therapy, age, age of diagnosis, height, weight, and presence of spontaneous puberty on bone density measurements at the different sites. $P < 0.05$ was considered to be significant. Sigma Stat for Windows version 2.03 statistical software was used (SPSS, Chicago, IL).

Results

Age, height, and body mass index were similar in the two groups (Table 1). Ethnic makeup of the two groups was identical, with 22 Caucasian and one Hispanic per group. There was no significant difference between the two groups in proportions of subjects with a history of androgen use, spontaneous puberty, current continuous estrogen use over the last 2 yr, thyroid hormone use, or a 45,X karyotype (Table 1). There was a significant difference in the age of diagnosis of TS, with nontreated group mean age of diagnosis of 13.6 ± 6 yr, whereas the GH-treated group's mean age of diagnosis was 3.7 ± 5 yr ($P < 0.0001$). The GH-treated group began treatment at 9.4 ± 3.6 yr and continued for 5.0 ± 2.1 yr. The usual GH dose was in the recommended range for TS, 0.3–0.38 mg/kg·wk given as daily injections. Eight subjects were still receiving GH at the time of the study.

Bone size is an important determinant of areal BMD and may be affected by GH, so we have reported the area of each of the measured sites (Table 1). The area of the one third radius (cortical bone) was approximately 8% larger in the GH-treated group ($P = 0.03$), but no other bone regions were significantly different in area between the two groups. However, to adjust for any differences in bone size that might confound the areal BMD data, we compared BMAD as well as BMD for each site. There was no significant difference in the two groups in BMD or BMAD at the wrist, hip, spine, or whole-body BMC (Table 1). Furthermore, a best subset regression analysis in a linear model including independent variables of age, age at diagnosis, height, weight, presence of spontaneous puberty, and GH use confirmed that GH use did not contribute to variation in BMD at any of the sites we measured.

We did not find difference in the prevalence and incidence of fractures between the two groups (Table 1). Most of the fractures were appendicular, and none was osteoporosis related.

Discussion

Our results suggest that GH therapy for a mean duration of 5 yr has no permanent effect on the bone density of girls

TABLE 1. GH therapy and BMD in TS

	GH therapy		*P*
	Yes (23)	No (23)	
Age [yr (range)]	21.5 ± 9.4 (7–35)	21.7 ± 9.4 (7–37)	0.95
Height (cm)	145.2 ± 10.9	143.3 ± 12.3	0.58
BMI (kg/m^2)	24.3 ± 6.0	24.2 ± 4.8	0.97
45,X karyotype in >90% of the cells	14/23 (61%)	9/23 (39%)	0.24
Spontaneous puberty (n, %)[a]	3/14 (21%)	8/15 (53%)	0.09
On HRT (n, %)[b]	15/16 (94%)	14/16 (89%)	0.62
On thyroid replacement (n, %)	7 (30%)	4 (17%)	0.32
History of androgen therapy (n, %)	4 (17%)	2 (9%)	0.42
RAD-1/3 area (cm^2)	2.51 ± 0.27	2.38 ± 0.29	0.034
RAD-1/3 BMD (g/cm^2)	0.56 ± 0.08	0.55 ± 0.08	0.67
RAD-1/3 BMAD (g/cm^3)	0.22 ± 0.03	0.23 ± 0.02	0.27
RAD-UD area (cm^2)	2.76 ± 0.41	2.82 ± 0.56	0.67
RAD-UD BMD (g/cm^2)	0.380 ± 0.077	0.378 ± 0.083	0.89
RAD-UD BMAD (g/cm^3)	0.141 ± 0.036	0.137 ± 0.035	0.73
AP spine L1–L4 area (cm^2)	45.91 ± 7.12	46.25 ± 7.34	0.82
AP spine L1–L4 BMD (g/cm^2)	0.82 ± 0.14	0.80 ± 0.16	0.41
AP spine L1–L4 BMAD (g/cm^3)	0.120 ± 0.019	0.117 ± 0.019	0.41
FN area (cm^2)	4.56 ± 0.58	4.47 ± 0.81	0.33
FN BMD (g/cm^2)	0.67 ± 0.10	0.66 ± 0.09	0.79
FN BMAD (g/cm^3)	0.148 ± 0.024	0.152 ± 0.028	0.61
Total hip area (cm^2)	29.32 ± 5.03	28.61 ± 5.75	0.28
Total hip BMD (g/cm^2)	0.73 ± 0.12	0.74 ± 0.11	0.66
Total body BMC (g)	1402 ± 347	1425 ± 424	0.94
Fracture prevalence (n, %)	7 (30%)	5 (22%)	0.75
Fracture incidence (per 100 TS patients year)	2.2	1.0	0.13

ANCOVA (continuous data), with adjustment for age, height, and age of diagnosis of TS, and Z-test for comparison of proportions. Data are means ± SD unless specified otherwise. BMI, Body mass index; HRT, hormone replacement therapy.

[a] Subjects 16 yr and older.

[b] Subjects 16 yr and older who have been taking HRT continuously for the last 2 yr.

and women with TS. This was true for skeletal sites with predominantly cortical bone (proximal one third radius and FN as well as sites with predominantly trabecular bone (lumbar spine and RAD-UD). This lack of GH effect was evident even after taking into account potentially confounding factors such as age, age of diagnosis, spontaneous puberty, and height/weight. GH-treated subjects had significantly larger bone area at the RAD-1/3, which means wider radius shaft, but no apparent difference in bone size of other skeletal sites. In addition, GH therapy did not translate into fracture risk reduction at that relatively young age.

We (9) have previously reported negative effect of GH treatment on cortical BMD at the radius in young women with TS. The discrepancy with our current report may be explained by the fact that our previous study was not designed to look for GH effect on the BMD and included only 10 women who had received GH that were not matched by age and estrogen therapy to those who did not receive GH.

GH is reputed to enhance bone mineralization during normal pubertal development, and GH-deficient individuals exhibit reduced BMD (12). GH treatment is reported to increase vertebral BMD in children with idiopathic short stature (13). Data regarding the effect of GH treatment on skeletal mineralization in girls and women with TS is limited. A longitudinal dose-response study evaluated phalangeal BMD in 68 Dutch girls with TS undergoing long-term treatment with GH by using radiographic absorptiometry (4). The authors found that, before treatment, girls with TS had normal cortical and trabecular BMD compared with healthy controls (Z-score close to 0), which increased significantly after 7 yr of GH treatment to reach a Z-score close to 1.0. The authors also found that the increase of BMD was mainly in the cortical bone, and the highest dose of GH caused the highest increment of BMD. This study was designed as a dose response, and there was no placebo control group; therefore, it is unknown whether the increase in BMD was caused by GH. In addition, it is unknown whether changes in phalangeal BMD reflect changes in other skeletal sites. Neely *et al.* (5) measured lumbar spine BMD and BMAD in girls with TS using dual-photon absorptiometry. Sixteen of these girls were treated with GH for a mean duration of 3.2 yr. Compared with age-matched normal girls, the TS group had normal BMD at lumbar spine, and compared with girls matched by pubertal status and/or bone age the TS group had increased BMD and BMAD. The authors concluded that the improved BMD in girls with TS could be a result of GH treatment, although in the absence of a control group that did not receive GH this is entirely speculative. In a longitudinal study of 18 girls with TS, ages 4–17 yr, Shaw *et al.* (6) also found normal BMD at lumbar spine when compared with weight- and pubertal status-matched normal girls. Twelve of their patients were receiving GH, and six were not. In a 2.5-yr follow-up, the authors did not find any influence of type of therapy (GH, ethinylestradiol, or combination) on the lumbar spine BMD and did not find significant relation between the dose of GH and the 2-yr BMD increment. The small number of patients who received GH, as well as the short period of follow-up, precludes any interpretation of these negative findings. Carrascosa *et al.* (7) measured, by DXA, lumbar spine areal BMD of 37 adolescent and young adults with TS; 11 of these had received GH therapy. The authors found no effect of GH treatment on spine BMD, irrespective of the presence or absence of spontaneous puberty. The small number of subjects who had received GH therapy and stratification by spontaneous and induced puberty in this study likely attenuates the statistical power to detect significant difference between the groups. Another very recent study reported no effect of GH therapy on lumbar spine BMD in a small group of Japanese women with TS (nine of 16 treated with GH) but found lower total body BMC in the GH-treated group (8).

Because GH treatment is demonstrated to increase height in girls with TS (2, 3), it is not feasible to conduct a randomized, placebo-controlled trial of GH effects on BMD in this disorder. Thus, the present cross-sectional and case-controlled study with a fairly large subject number may be useful to address this issue. We had statistical power of 0.8 to detect difference between 8 and 22% in the BMD/BMAD measurements at the different sites and between 10 and 17% in the measurement of the different bone areas. There are several limitations to this approach, however. The dose of GH and duration of treatment were not controlled in our study group, although GH treatment clearly had demonstrable effects, as there was a clear increase in bone size of the forearm in the treated group. Another major weakness is the lack of randomization to GH treatment, such that a selection bias may exist, *e.g.* treatment for only the shortest children. The major reason for absence of GH treatment in our study group was a delayed diagnosis of TS. According to our study subjects and their families, they were very short as children, but for a variety of reasons the diagnosis of TS was not made until later in life, usually on the basis of primary or secondary amenorrhea. Yet, we do not have retrospective data on height for both groups, and it remains possible that the early diagnosis in the GH-treated group was due to a more severe TS phenotype; therefore, this group may have been shorter and had a lower BMD before treatment that improved as a result of treatment.

With the above-mentioned limitations in mind, our data suggest that GH treatment does not increase BMD at the lumbar spine, hip, or wrist in patients with TS.

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